United States Environmental Protection Agency **Office of Research and Development**

National Exposure Research Laboratory
Research Abstract
Government Performance Results Act Goal: Safe Food

Significant Research Findings:

Immunochemical Methods

Scientific Problem and Policy Issues

The immunochemistry program of the U.S. Environmental Protection Agency's (EPA) National Exposure Research Laboratory's (NERL) Human Exposure Research Branch is addressing the need for rapid, cost-effective monitoring methods to assess human exposures to low levels of environmental contaminants such as pesticides. Immunochemical methods such as immunoassays can provide information regarding the presence and concentration of contaminants that might impact human health and the environment. One way to reduce uncertainties in the determination of low-level, non-occupational exposures is to better characterize potential routes of exposure through extensive environmental monitoring and dietary studies. The information from these assessment studies is then used to ultimately mitigate or reduce exposures. A large number of multi-media samples (e.g. air, water, soil, dust, and food) are required for sampling and laboratory analysis, which can often be slow and expensive; however, time and budget constraints limit the number of samples that can be analyzed. Since analytical methods are the foundation of the overall exposure assessment and risk management process, reliable and cost-effective monitoring methods such as immunoassays are key to safe guarding human and environmental health.

Research Approach

The need for new methods is determined through extensive literature searches and discussions with Program Offices and NERL researchers responsible for measurement studies. After target chemicals and matrices have been identified, immunoassays are either developed or adapted for particular applications. The experimental design and statistical approach is specific for each chemical and is dependent upon the data quality objectives of the particular study. The guidance in "A User's Guide to Environmental Immunochemical Analysis" (EPA/540/R-94/509) is followed throughout the methods development process. Immunoassays for parent compounds, environmental degradation products, and biomarkers of exposure are ultimately configured for appropriate environmental and biological matrices to support an integrated multimedia approach to environmental monitoring

and risk assessment. Research results are published in the peer reviewed literature and presented at scientific meetings.

Results and Implications

Immunoassays to detect the pesticide chlorpyrifos (O,O-diethyl-O-[3,5,6-trichloro-2-pyridyl]-phosphorothioate and its metabolite TCP (3,5,6-trichloro-2-pyridinol) in food, track-in dirt, house dust and urine have been developed. The assay format is an indirect competitive enzyme-linked immunosorbent assay (ELISA) which utilizes a color endpoint. Based on a 96-well microplate format, the ELISA has a high sample capacity. A streamlined approach for the immunoassay determination of chlorpyrifos and TCP residues in various food matrices was developed to analyze samples from dietary exposure surveys. Sample preparation is based on a sonic extraction followed by dilution to reduce interfering components. Recoveries range from 74-95% for various food commodities. Cleanup by solid phase extraction can be employed for more difficult food matrices. Samples can also be prepared using accelerated solvent extraction or supercritical fluid extraction techniques prior to detection by ELISA. Chlorpyrifos levels of 5-400 ppb can be determined based on a 10 g sample. Initial evaluations indicate the ELISAs perform favorably with gas chromatography/mass spectrometry (GC/MS) procedures; however, for the ELISAs, sample preparation is minimal with a high sample throughput and lower cost.

Research Collaboration and Publications

This immunochemistry program is conducted in-house with technical support through the Senior Environmental Employee Program (Cooperative Agreement #826228). An extramural component is conducted through Battelle, Columbus, Ohio (Contract #69-D-99-011). Examples of recent publications and presentations from this program include:

Chuang, J.C., Hart, K., Chang, J.S., Boman, L.E., Van Emon, J.M., and Reed, A.W. "Evaluation of Analytical Methods for Determining Pesticides in Baby Foods and Adult Duplicate Diet Samples," *Analytica Chimica Acta*, (accepted 2002).

Van Emon, J.M. "Immunochemical Applications in Environmental Science," *Journal of AOAC International*, Vol. 84, No. 1, (2001), 125-133.

Van Emon, J.M., Brumley, W.C., Reed, A.W., and Chuang, J.C., "Human Exposure Assessment Using Immunoassay," presented at the 221st American Chemical Society, San Diego, CA, April 1-5, 2001.

Van Emon, J.M., and Reed, A.W., "Immunoassay Analysis for Chlorpyrifos in Foods," presented at the 37th Pesticide Residue Workshop and Florida Foodborne Pathogen Analysis Conference, St. Petersburg Beach, FL, July 16-21, 2000.

Chuang, J.C., Pollard, M.A., Misita, A.M., and Van Emon, J.M., "Evaluation of Analytical Methods for Determining Pesticides in Baby Food," *Analytica Chimica Acta* 399, (1999) 135-142.

Van Emon, J.M., Gerlach, C.L., Reed, A.W., and Hardwick, B.C., "Foliar Dislodgeable Residue Analysis: A New Scientific Approach to a Regulatory Concern," *Food Technol. Biotechnol*. 36, (1998),119-124.

All journal articles and abstracts from the immunochemistry program were reviewed and approved in accordance with ORD's scientific peer review procedures.

Future Research

The TCP and chlorpyrifos ELISAs will be used to analyze samples from on-going and planned NERL exposure studies. Additional evaluations with GC/MS will be conducted based on the parameters of precision, accuracy, sample throughput and cost. ELISA development for compounds identified through the literature survey recently completed will be initiated. Probable candidates include the pyrethroids for various food matrices as well as their urinary biomarkers of exposure. Selective antibodies will also be configured for immunoaffinity chromatography columns. Immunoaffinity chromatography will be used for sample preparations prior to ELISA detection and coupled on-line with liquid chromatography/MS. As the need for rapid, low-cost analytical methods continues, immunochemical methods will be developed and applied.

Contacts for Additional Information

Questions and inquiries can be directed to:

Jeanette M. Van Emon, Ph.D.
US EPA, Office of Research and Development
National Exposure Research Laboratory
Las Vegas, NV 89193-3478

Phone: 792/798-2154
E-mail: vanemon.jeanette@epa.gov

Federal funding for this research was administered under EPA cooperative assistance agreement #826228 with the Senior Environmental Employee Program and under EPA contract #69-D-99-011 with Battelle, Columbus, Ohio.